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THE RHYTHM OF IMMUNITY AND SUSCEPTIBILITY OF FERTILIZED SEA-URCHIN EGGS TO ETHER, TO HCl, AND TO SOME SALTS.

E. G. SPAULDING.

INTRODUCTION.

The experiments described in this paper were undertaken during the summer of 1902 at the Marine Biological Laboratory upon the suggestion of Dr. A. P. Mathews; their publication has been delayed because of the pressure of other work and of the desire to, if possible, get beyond their mere description to their meaning. This end is believed to have been attained in connection with the working out of a synthesis of the artificial parthenogenetic methods, the detailed results of which attempt appear in a preceding paper.¹ The effectiveness of all these methods and so the special physico-chemical result of normal fertilization and the nature of cleavage processes can, it is believed, be explained from a unitary standpoint, viz.: if it is considered that in the process of cleavage an average decrease in surface tension takes place as a result of the equilibrating of a potential difference between osmotic pressure and surface tension, accompanied by such electrolytic changes as cause the constricted form. That this average decrease in surface tension takes place is a necessary inference from the change from the approximately spherical to the constricted form of the egg at cleavage, for this means an increase in surface. It carries with it, therefore, the decrease in that potential, osmotic, which opposes surface tension in direction. The preceding cause in these events is the creation of a potential difference by first increasing the osmotic pressure, which is done artificially by each of the parthenogenetic methods. Accordingly with the equalization of this difference, caused, *e. g.*, by a splitting up of colloidal particles or molecules, there is in the case of eggs of marine forms an absorption of water. Both of these

¹ Spaulding, E. G. "The special physics of segmentation as shown by the synthesis, from the standpoint of universally valid dynamic principles, of all the artificial parthenogenetic methods." BIOLOGICAL BULLETIN, February, 1904.

last events either alone or together may constitute what is termed liquefaction.

The results of the experiments given below can, it is believed, be interpreted in agreement with this view of segmentation. They were undertaken primarily as an extension of experimental work which had already been done on the action of various chemical compounds on protoplasmic bodies, but were limited to the study of such action on the eggs of *Arbacia* at successive periods after fertilization.

The existence of a rhythm of immunity and susceptibility has been shown already by Lyon in studying the effect of KCN and of lack of oxygen upon the fertilized eggs and embryos of the same form,¹ and it has been found also that many eggs do not segment at all in the absence of oxygen, notably *Arbacia*² and *Ctenolabrus*.³ Lyon also found this summer that *Arbacia* eggs required more oxygen during precleavage and gave off more CO₂ during cleavage than at other times.⁵

From these results it may be inferred in analogy to a large number of instances well known in chemistry that at least the *ultimate* effect of oxygen on the processes conditioning cleavage is the causing of analytic chemical changes; *i. e.*, fermentation, one might say, occurs and CO₂ is given off, as Lyon found. Previous to this, however, synthetic processes may take place which in turn as certain preferments become active⁴ give rise to molecular splitting. The result of such analytic change is that increase in osmotic pressure and therefore the creation of that potential difference between it and the surface tension which we have found to be necessary and in the equalization of which both potentials decrease, water is absorbed, and the egg cleaves.

The hypothesis to be deduced from this and which might serve as a guide in our experimentation is that any method either (1) of preventing this necessary preliminary increase in osmotic pressure, or of compensating it after it has been created, or (2) of increasing it beyond a certain point, will tend to do away with

¹ Lyon, E. P., *American Journal of Physiology*, VII., 1.

² Lyon, *loc. cit.*

³ Loeb, J., *Archiv für die gesamte Physiologie*, 1895, LXII.

⁴ Cf. Hofmeister, "Chemische Organisation der Zelle."

⁵ Personal communication.

the event of cleavage. To the first two possibilities correspond the effect respectively of lack of oxygen and the use of strongly hypertonic solutions on the fertilized egg; to the second method our own results with ether, HCl, etc. From this hypothesis it can also be reasonably inferred that the nearer to the point of termination of the preparatory process that either method is used, so much the less will its effect in general be, and this supposition is again confirmed by experimental results.

Lyon¹ found that the effect of KCN on the fertilized *Arbacia* egg, taking, *e. g.*, various strengths of a titrated solution mixed with sea water, was the indication of "successive stages of relatively high and low resistance in each cleavage." Putting the eggs into the solution at successive five-minute periods after fertilization and allowing them to remain perhaps one hour, then washing and removing to sea water, he found that "there is a stage about ten or fifteen minutes after fertilization when the egg is especially susceptible to KNC." "Again soon after the first cleavage comes a second stage of small resistance; a third follows the second division." "The resistance of the egg to KNC increases up to a *maximum* up to the time of *separation into* the two cells." "The effect of KNC is the same as lack of oxygen."

In interpretation of these results Lyon says that the processes dependent upon oxygen seem to begin about 10-15 minutes after fertilization, for if they are inhibited the egg does not segment and they recur at each segmentation. To identify them with the morphological processes of the splitting and separation of the chromosomes or with the dissolution of the nuclear membrane seems to him to be impossible, for these occur too late to be directly affected. Wilson and Matthews,² he says, mention however two processes which occur sufficiently near to the susceptible stage to be worthy of consideration in this respect. One is the growth and division of the sperm aster, the other the growth of the nucleus. From the part which in order to explain the constricted form of cleavage³ must be attributed to, as played by each of these processes, the supposition that they are affected by a lack of oxygen, by KCN, etc., receives confirmatory evidence.

¹ *Loc. cit.*

² *Journal of Morphology*, 1895, X., p. 319.

³ Lillie, R. S., BIOLOGICAL BULLETIN, IV., March, 1903; and *Am. Jour. of Physiology*, VIII., 4, Jan. 1903.

But furthermore, whatever the morphological elements may be, it must also be admitted that in the processes leading up to and culminating in cleavage, we are dealing with chemical and electrolytic and consequently also with osmotic phenomena, coexisting with those of surface tension. That these first two which condition the other two are, however, not uniform, but, rather, are varying, *i. e.*, rhythmical, during that period must be admitted to explain the observed rhythm in morphological changes.

The experiments herein described serve the purpose then of testing the above-mentioned hypothesis of the existence of a liquefaction during the event of cleavage, and of a rhythm of increasing immunity up to and of marked *susceptibility during* that time. To this end use was made of ethyl ether, HCl, KCl, NaCl and sodium citrate solutions.

THEORIES OF THE NATURE OF THEIR ACTION.

From the position that has been taken in this and a previous paper that, inasmuch as in protoplasm we are dealing with colloidal (probably also electrolytic) particles in solution, we therefore in segmentation necessarily have to do ultimately with the relations of two kinds of energy, osmotic and surface, and that the cleavage process itself depends upon the existence of an uncompensated potential difference between these, from this it follows that this potential difference might be caused *in either of two ways*, viz., at the same time that either one is kept constant, by changing the other, *i. e.*, either increasing the osmotic or decreasing the tension factor, the former being identical with the energy of the particles in solution, the latter with that of the solvent. The theories also which we find advanced in order to explain the nature of *stimulation* seems to us to be in complete agreement with this view. For example, we find the statement that "stimulation consists in the precipitation, *i. e.*, gelation, of colloidal particles and is due in the case of positively charged particles to the negative ions, *i. e.*, to the charge;¹ the inhibition of this stimulation, *i. e.*, what in some cases is termed poisoning, to the positive ions"; for negative particles the converse would hold

¹ Mathews, A. P., "The Nature of Nerve Stimulation, etc.," *Science*, March 28, 1902.

true. In any case the osmotic pressure would necessarily be affected. Both the stimulating and poisoning effect have furthermore been correlated with the valency.¹ In the case of a compound of anion and kation, both of which are monovalent, like NaCl which does and KCl which does not stimulate easily and when in both therefore the charges might seem to offset each other, the stimulating effect, *e. g.*, on the nerve has nevertheless been said to be due to the "overbalancing" of the kation by the anion, and conversely for the inhibitory effect. This of course is not real explanation unless the difference in effect can be correlated with a difference in some such quality as velocity of diffusion or solution tension, and Mathews has this summer shown that the poisoning qualities of the metals and non-metals as well are in fact a function of this latter. Some ground for this "overbalancing" effect seems to be furnished by the fact that in the case in which *e. g.*, a divalent anion is combined with a monovalent kation (2) a greater stimulating effect is observed. Thus KCl does not stimulate the nerve at its osmotic pressure, K_2SO_4 does occasionally, K_3 citrate stimulates in solutions of a gram molecule to 22,000 c.c. H_2O . But even here the number of opposing charges is the same. The kations therefore differ in some way other than in their mere number of charges. This must also hold true of the anions because of the increasing stimulating effect on the nerve of NaCl, NaBr, NaI and NaFl. The suggestion has been made that the difference in effect when the charges are the same in number is due to a difference in the translatory path of the charge around the atom; but as a cause for this latter difference must in turn be assigned the admission of an ultimate difference in the atoms themselves would seem to be necessitated.

While the salts therefore seem to affect the colloidal particles directly, the known inhibitory action of the anæsthetics would accordingly have to be identified with a direct effect on the solvent and so only indirectly on the solute. Thus it may be considered that the anæsthetics as being better solvents in most cases than water have the same effect on colloidal particles as do like charges, which repel; therefore they increase the osmotic

¹ Loeb, *Archiv für die gesch. Physiologie*, Bd. 88.

pressure. It is believed that on this basis the varying effect of ether on the eggs of *Arbacia* at successive periods after fertilization can be explained.

EXPERIMENTAL.

1. *The Effect of Ethyl Ether.*

The general methods of experimentation may be outlined as follows: First, a slightly supersaturated solution of ether in sea water was prepared, kept tightly corked, and when used a portion was drawn from the bottom, thus ensuring a saturated solution. The eggs were fertilized in the usual way, good lots from among a number being selected. At successive periods these eggs were transferred to staining jars containing 50 c.c. of the solution used. The strength of the solution actually used was accurately controlled by starting with twice that strength and then diluting exactly one half, in part with the sea water necessary for transferring purposes. The eggs were allowed to stand in these *covered* jars for the length of time selected; the solution was then carefully drawn off; the eggs were thoroughly washed twice with sea water, which was again added, and given time to develop. In important experiments the lots were each observed twice. All the experiments were conducted at the room temperature, about 20° C.

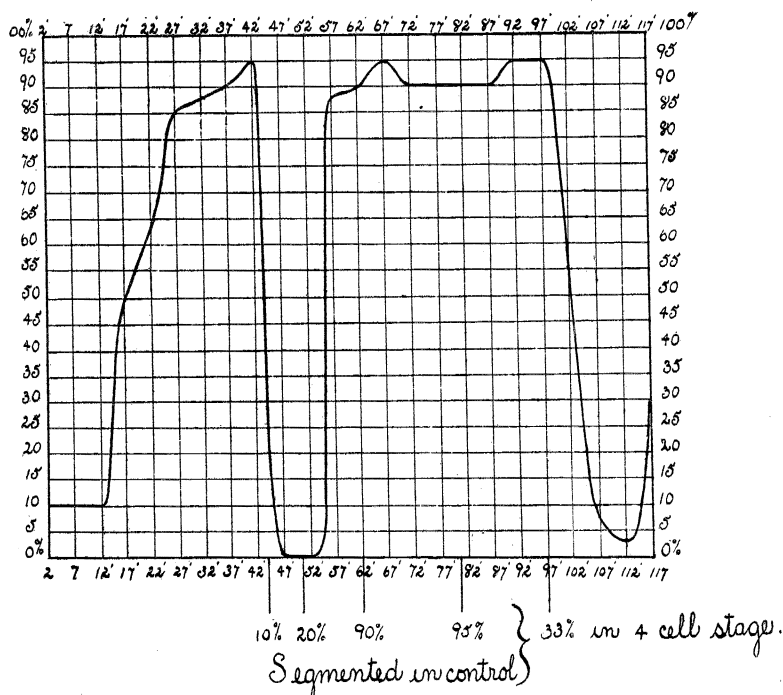
The practical problem presented was to get such a strength of solution and time of exposure that of the eggs transferred at the various periods after fertilization some would be stopped in their development, others not. For each value of the one factor there would probably be a corresponding value of the other, the product being a constant. An abstract record of what were essentially preliminary experiments is given on the next page.

From these results it seemed that the correct relation between the two variables, time and solution strength, had been found; accordingly in the next experiment a one sixty-fourth saturated ether solution was used for 25', which gave very satisfactory results. These are tabulated and plotted on page 008.

In the plotting of the curve of these results the abscissæ represent the times of transferral after fertilization, the ordinates the per cent. of swimmers found by as accurate observation as possible.

Exper.	Strength of Solution.	Period After Fertilization.	Time of Exposure.	Observation.	Control.
I. July 24.	Saturated.	Every 15'. 8 lots.	1 hour.	All dead in the stage of treatment.	Well developed
III. July 26.	$\frac{1}{4}$ and $\frac{1}{8}$ sat. sol. by diluting with sea water (same lot of eggs).	Just before and after each cleavage.	Various.	All dead.	Well developed
IV., A. July 29.	$\frac{1}{8}$ sat. sol.; one lot of eggs for A, B, and C.	At "critical" periods, <i>i. e.</i> , just before and after each cleavage, as below in B.	30'	All dead.	Control good, regular.
IV., B.	$\frac{1}{8}$ sat. sol. Lot (1).	15 minutes. (Lyon's 1st critical point.)	45'	50% unsegmented, but pigmented and swollen, some in 4 and 8 cell stage; 20% swimming.	"
	(2)	50', 1st. cleavage just beginning.	30'	80% dead, swollen, pigmented, 20% swimming.	"
	(3)	1 hr. 10', toward end of cleavage.	30'	87% dead, irreg. segment., some in 16 cell stage 13% swimming.	Segmentation going on.
	(4)	2 hrs., during 2d. cleavage.	30'	All stopped in 4 cell stage.	Control good.
	(5)	2 hrs. 40', after 2d. cleavage.	30'	All dead.	
IV., C.	$\frac{1}{8}$ sat. sol. (1)	15', Lyon's critical point.	45'	8 hrs. afterward all had segmented, 8, 16, 32 cell stage; 25% swimming.	Control good.
	(2)	50', cleavage just beginning.	30'	(Like C 1.)	
	(3)	70', toward end of cleavage.	30'	Nearly all swimming.	
	(4)	2 hrs., during 2d. cleavage.	30'	2-32 cell stages present; decomposed and pigmented; $\frac{1}{5}$ swimming.	
	(5)	2 hrs. 40', after 2d. cleavage.	30'	$\frac{1}{5}$ swimming.	

Exper. V. July 30.	Solution 1/64 Sat.	Time After Fertiliza- tion.	Exposed to Solution.	Observation.	Control.
Lot I		2'	25'	20% swimming.	
" 2		7'		20 "	
" 3		12'		20 "	
" 4		17'		50 "	
" 5		22'		62 1/2 "	
" 6		27'		85 "	
" 7		32'		88 "	
" 8		37'		90 "	10% in 2-cell stage, 44' after fertilization.
" 9		42'		95 "	
" 10		47'		0 "	20% segmented.
" 11		52'		all dead, pigmented, and swollen.	20% "
" 12		57'		87.5% swimming.	
" 13		62'		90 "	90% "
" 14		67'		95 "	" "
" 15		72'		90 "	
" 16		77'		90 "	
" 17		82'		90 "	95% "
" 18		87'		90 "	
" 19		92'		95 "	
" 20		97'		95 "	33% in 4-cell stage.
" 21		102'		55 "	
" 22		107'		7.5 "	
" 23		112'		2 "	
" 24		117'		30+ "	



The character of this rather remarkable curve is obvious. Up to within twelve minutes after fertilization the resistance remains the same, but from this point on it gradually rises up to either *just before* or the *beginning* of the first cleavage ; during the early part of cleavage it falls to zero, with a sharp rise afterwards and a fall at the second segmentation.

The more important question however is to get at its meaning. To get at this we take, corresponding to the general rise in immunity up to the time that cleavage is beginning, the greater demand for oxygen, established by Lyon in his work this summer. This might mean in view of the fact that either at least just preceding or *for some time* before cleavage an increase in osmotic pressure must take place, as we have shown, either one of two things to account for this, viz., either that the oxidation process is at first synthetic and subsequently determines analytic events ; or that it is analytic from the start. Also to be correlated with this is the known effect of ether as a better solvent than is water. This is identical with its causing a greater degree of solution and consequently an increased osmotic pressure. The inhibiting effect of the ether on the eggs at the critical period in the above curve may be ascribed then, we believe, to its *augmentation* of the normal predominance in osmotic pressure at that time, *i. e.*, to its increase of that difference of potential in the direction of pressure-tension *necessary* for cleavage. Accordingly in the equalization of this *augmented* potential difference the eggs would be expected to increase in size more than usual in their attempt to divide, and this is confirmed by the *observed swollen* appearance, even when as in some cases division takes place once and then stops.

This increasing immunity up to the maximum can be explained then in two possible ways. If synthetic as simple oxidation processes precede the analytic then during that period there is something to *oppose* the dissolving effect of the ether ; but since this *opposition* would seem to exist *equally* all through the precleavage period, the *rise* in immunity would be hard to account for in this way. On the other hand if analytic processes take place from the start as a result of the use of oxygen then the *longer before* cleavage that the exposure to ether is made the *greater* should be

its effect in augmenting the normally occurring increase in the pressure, and the point of greatest susceptibility would be at such points and also at that of the normal maximum pressure, viz., just before or during cleavage. This explanation therefore accords best with the sharp rise in the curve, and is supported also by the evidence from the parthenogenetic methods for *Arbacia*, in which the pressure is first increased, *sometime before* the cleavage, which takes place after the return from the hypertonic solution to the sea water.

The characteristics of the above curve were confirmed in general by *Experiment IV.*, B and C, already presented, and more especially by three subsequent experiments, as can be seen from the following records :

EXPERIMENT VII.

August 22, 1/64 sat. sol. Time of exposure, 1/2 hr.

Lot.	Period After Fertilization.	Observation.	Control.
1	3'	66% unsegmented.	Middle point of segmentation.
2	19'	90% living.	
3	31'	90% "	
4	56'	95% dead, many in 2-cell stage, pigmented. swollen.	
5	63'	25% swimming.	During second cleavage.
6	79'	25% "	
7	96'	All dead in 4-cell stage.	
8	112'	" "	

Experiments VIII. and IX., August 23 and 29. Solution, one sixty-fourth saturated ether, one half hour exposure. Lot 1, one half hour after fertilization all living. Lot 2, during segmentation, *all dead* in 2-cell stage.

EXPERIMENTS WITH HCl.

Preliminary and theoretical. According to the views that we have previously discussed the hydrogen ion in the case of the nerve is held to inhibit the stimulating action of the chlorine ion, "overbalancing" this more than do either K, Li, NH₄ or Na. This may be due to the greater velocity of diffusion of H, which is 325, that of Cl 70.2 at 25° C.¹ On the other hand it has been supposed that the H ion brings about the parthenogenetic devel-

¹ Ostwald.

opment in *Asterias*. These two seemingly contradictory effects cannot, however, be so in reality and the difficulty may be done away with if it is borne in mind that the effect depends as much on the character (electronic) of the colloid as on the agent. For on positively charged particles the H ion would have a repelling, *i. e.*, dissolving ; on negatively charged, the opposite effect.

If all the protoplasm of the *Arbacia* egg was uniformly positive just prior to or during segmentation it accordingly might be deduced that H ions would have the same effect in increasing the osmotic pressure as does ether, and so of inhibiting development. Lillie,¹ however, has shown that at cleavage the cytoplasm is markedly electropositive, the nucleus negative. Accordingly at that time he holds that the periphery repels the free kations within the egg and the nucleus the anions, so that the kations then predominate at the center, the anions at the periphery. As like charges repel each other, this is made to account for the constricted form of the egg at cleavage. In agreement with this view the effectiveness of the H ions, in parthenogenetic methods, *in the environment* might be considered to be due to their induction of a predominance of negative charges at the surface and this in turn of positive charges at the astral centers. If this be so, however, then other kations ought to have the same effect ; but they do not. This indicates a specific action by the H ion, which it might have in accordance with its high diffusion velocity. It alone might therefore be considered to penetrate the egg membrane because of its and the latter's definite chemical make-up ; yet there remain difficulties even here in explaining why it should do this, since, if, at least before cleavage, the membrane and cytoplasm are themselves positive they would tend to repel the H ions rather than to attract them. Only provided the surface were negative from the start could the attraction be explained. If, however, the egg, when *just about* to divide, were put into such a medium of H ions, it is reasonable to suppose that since the surface at least then is negative these might be attracted ; but again it is difficult to understand how they can go further, since the cytoplasm is even yet positive. However, if they succeed

¹ Lillie, R. S., *Am. Jour. of Physiology*, VIII., IV., and BIOLOGICAL BULLETIN, IV., 4.

in acting on the cytoplasm the effect would be a repulsion of its particles and an increase in pressure, the same as that of ether. Accordingly it would *a priori* be probable that the period of least immunity to HCl would coincide with that of the greatest normal pressure, viz., just before cleavage.

EXPERIMENTAL.

Two preliminary experiments with seventeen different strengths showed that the proper strength of solution, for an exposure of one half hour, was between a $\frac{1}{400}$ and $\frac{1}{500}$ normal HCl solution. Accordingly a $\frac{1}{450}$ *n* solution was next tried with the following results :

EXPERIMENT IV.

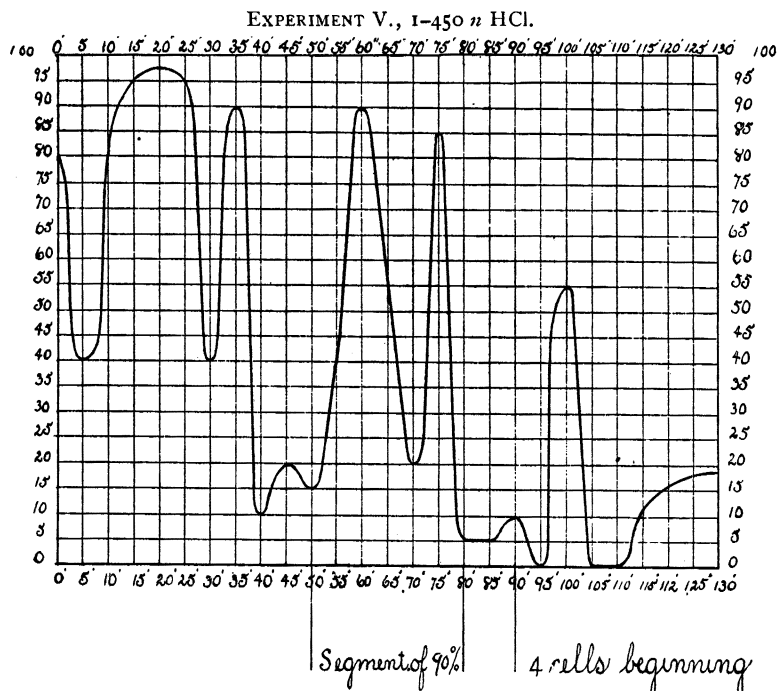
August 3, $\frac{1}{450}$ *n* HCl solution (by diluting with sea water); three series, 25', 30' and 35' exposure.

Lot.	Time After Fertilization.	Series A, 25'. Per Cent.	Series B, 30'. Per Cent.	Series C, 35'. Per Cent.	Control. Per Cent.
1	1'	No segmentation at all. ¹	A few swimmers.	A few swimmers.	
2	16'	50 swimming.	80 swimming.	50 swimming.	
3	31'	30 blastulæ, stopped. Quite undeveloped and disintegrated.	Like A ³ .	Gastrulæ. Like A ³ .	
4	41'	80 swimming.	40 swimming.	40 swimming.	Segmentation began here.
5	46'	All dead in 1- and 2-cell stage.	Like A ⁵ .	Like A ⁵ .	Segmentation going on.
6	53'	40 swimming.	20 swimming.	20 swimming.	} Toward end of cleavage.
7	59'	50 "	50 "	50 "	
8	77'	All dead in 2- and 4-cell period.	Like A ⁸ .	Like A ⁸ .	
					15 were in 4-cell stage.

August 5, *Experiment V.*, $\frac{1}{450}$ *n* HCl solution, used every five minutes for one half hour. The results are plotted on the next page, the times after fertilization being the abscissæ, the percentage of swimmers the ordinates, and are in general confirmation of the previous experiments with HCl.

Two other experiments were made with HCl, which gave a general confirmation of the two described. These all agree in giving a rise in immunity soon after fertilization, then a fall at

¹The difference between A¹, B¹, and C¹ may be due to the effect of the necessary manipulation on the eggs transferred so soon after fertilization, viz. A¹; B¹ and C¹ were necessarily transferred a little later. Cf. Mathews, *American Journal of Physiology*, VIII., IV., "Importance of Mechanical Shock on Protoplasmic Activity."



30', followed by a second rise and a second fall respectively before and during segmentation. This fall is repeated at the beginning of the second cleavage.

Comparison of this with the 'ether curve' however shows a difference in respect to this 'thirty-minute fall,' and that this should be so is to be expected from the possibility of the agent here used (HCl) directly affecting the colloidal particles as well chemically as electrically, as the evidence for a specific H ion action indeed indicates; while in the case of ether the solvent alone is first concerned.

Assuming that the H ions in some way penetrate the egg, the increase in osmotic pressure resulting therefrom might be connected with the dissolution of the nuclear membrane, which occurs at about the time of the first, the 30' fall,¹ in such a way that an abnormal increase in pressure results from both, which inhibits development. This however could not be due alone to the electronic action of the ions for were this so ether also

¹ Mathews and Wilson, *loc. cit.*; cited by Lyon.

would have the same effect in increasing the pressure abnormally and causing a fall in immunity at the same time, which it does not do. Hence from this, together with the evidence, in other instances, of H acting differently than do other ions, and the probability that the dissolution of the nuclear membrane is caused or accompanied by chemical changes, it may be inferred that the H ions have a specific chemical effect, which, in addition to their electronic action, accounts for the fall in immunity at about 30' after fertilization.

Both series of experiments agree, however, in making the period of greatest susceptibility just at the beginning and during the earlier part of cleavage, though for the HCl this drop seems to come somewhat earlier than for ether. The explanation for this effect of HCl we believe to be essentially the same as for that of ether, viz., that the H ions in some way penetrating the membrane cause a repulsion of at least the cytoplasmic particles, thereby *augmenting* the normally increased pressure to such an extent that further development is inhibited.

EXPERIMENTS WITH KCl, NaCl AND Na CITRATE.

The acceptance of a specific action for the ions is, of course, by implication not limited to H, but is quite as necessary for others, like K, Na, Ca, etc. For instance Loeb in one place holds that the kations (specific) and not the anions are poisonous,¹ for the reason that the newly fertilized *Fundulus* egg will develop in KCl but not in NaCl² and because, of fertilized *Arbacia* eggs in $\frac{5}{8}n$ NaCl only 10, 20, and in one case 50 per cent. began to segment, the majority stopping in the 2-cell stage, while in $\frac{5}{8}n$ KCl, 70–80 per cent. segment to 8 cells.³ Opposed to this view of Loeb's is that which ascribes toxic effects also to the anion, for, *e. g.*, NaCl, NaBr, NaI and NaF have a different poisoning effect. The two views, however, are quite compatible if the colloids affected are in the two cases of different sign. Loeb also finds that the toxic effects of Na salts is a function of the valency, increasing from the acetate to the citrate.⁴ Further-

¹ Loeb, *Am. Jour. of Physiology*, III., VII. and VI., VI. and *Archiv für d. ges. Physiol.*, Bd. 88, 1901.

² Loeb, *Am. Jour. of Physiology*, III., VIII.

³ Loeb, *Am. Jour. of Physiology*, III., IX.

⁴ *Am. Jour. of Physiology*, VI., VI.

more, as an illustration of the lack of a consistent theory here, are the views of Loeb, that the antitoxic as well as toxic effect is a function of specific kations, and of others that where the kations are toxic the anions are antitoxic. For instance, in 100 c.c. $\frac{5}{8}n$ NaCl + 8 c.c. $\frac{1}{6}\frac{1}{4}n$ CaSO₄ or Ca(NO₃)₂ 70 per cent. of *Fundulus* eggs develop, while if Na₂SO₄ be substituted they do not. Ca is therefore considered to be antitoxic to Na. Al₂Cl₃ and Cr₂(SO₄)₃ also have the same inhibitory effect on Na, but in smaller quantities. Loeb therefore concludes that the toxic and antitoxic effect of the ions is a function of their valence, but that only kations are poisonous. The necessity for such "balanced" solutions also holds good according to Loeb for muscle and for the contractions of *Gonionemus*; "margin and center must contain three ions, Na, K and Ca."¹

The opposite view is that in the instance of, *e. g.*, NaCl poisoning but KCl not, the difference in effect is due to a greater overbalancing by the Cl ion in one case than in the other.² The effect is due in any case to both the ions. Both may be either toxic or antitoxic according as the colloid is like or unlike in charge and the normal event is one of gelation or of liquefaction.

Accordingly if the normal progress of cleavage in *Arbacia* demands liquefaction, *i. e.*, increased pressure and absorption of water, any salt either preventing this by unlike charges or augmenting it by like beyond a certain point may be assumed to interfere with or inhibit division. And from the data at hand we may expect NaCl to do this to a less degree than KCl.

Experiment IV., 7 pts., $\frac{5}{8}n$ KCl sol. to 2 of sea water, the other conditions as in III., gave *exactly the same* results, and is tabulated on the next page.

Examination of this record makes manifest a decrease in immunity beginning 15 minutes before cleavage, much as with HCl, but reaching its *maximum during segmentation*. The same *explanation* of this effect that was made for HCl and ether holds good, we believe, here; but the thirty-minute fall obtained with HCl is not present in these series, which indicates a different specific chemical effect of H and of K ions. Two subsequent KCl experiments confirmed these results.

¹ *Am. Jour. of Physiology*, III., VII.

² Mathews, *Science*, March 28, 1902, and May 8, 1903.

EXPERIMENTS WITH KCl.

	Solution.	After Fertilization.	Exposure.	Observation.	Control.
Exper. I., Aug. 16. Lots I.-III.	$\frac{1}{3}n$.	At critical periods before cleavage. During cleavage, 66' after fertili- zation.	1 hour. 1 "	90-95 % swim- ming. 75% swimming.	Good.
Lot IV.					
Exper. II., Aug. 17. Lots I.-IV.	$\frac{5}{12}n$ KCl.	Before cleavage. During cleavage.	1 hour.	All dead in 2 cells. Dead.	Good.
Lot. V.					
Exper. III., Aug. 19.	8 pts. $\frac{5}{8}n$ KCl + 2 of sea H ₂ O in transferring.	every 5'	1 hour.		
Lot I.		5'		80% swimming.	80 % swim- ming.
" II.-VIII.		(10'-40')		80 "	
" IX.		45'		70 "	
" X.		50'		60 "	
" XI.		55'		50 "	
" XII.		60'		25 " Remainder dead and disinte- grated.	Segmentation began here, 60' after.
" XIII. and XIV.		65'-75'		5-10 % swim- ming, remain- der pigmented and disinte- grated.	

Experiment I., August 29, with NaCl, 15 pts. n to 1 of sea water, exposure one hour, at chosen critical periods. Observation showed a slight rise in immunity from 70 to 95 per cent. up to the beginning of segmentation, then a fall to 70 per cent. during that process, but as opportunity for further experimentation was lacking this result is hardly conclusive, though by itself it is confirmatory of the supposed greater immunity to NaCl than to KCl.

Experiment I., with the Na citrate, August 13; 3 series, viz., 2 c.c. of seven-fourths n Na citrate to 300, 400, 500 c.c. respectively of sea water, *diluted* one half at transferral. 15' periods, 1 hour exposure. Parallel observations showed that the eggs so treated were *ahead* of control both in beginning to segment

and in the number segmented at all cleavages. No period of susceptibility was indicated. This acceleration effect might according to the views above discussed be ascribed to the so-called "overbalancing effect" of the trivalent radical, but the experiment was not repeated because of lack of time.

SUMMARY AND CONCLUSIONS.

1. There is a pronounced *rise* in *immunity* of fertilized sea-urchin eggs to *ether* up to either *just before* or the *beginning* of segmentation; the exact point is impossible to determine owing to the unevenness of the cleavage. A *sharp decrease* then occurs, followed by a *sharp rise toward the end* of the cleavage. A repetition of this occurs at the second segmentation.

2. *Similar* changes are found resulting from the use of HCl, KCl, and NaCl with the difference that the fall in immunity comes somewhat earlier with KCl than with HCl and with this than with ether. All of these differ therefore from Lyon's interpretation of the susceptible points with KNC as occurring "after division," nor is a 15' period found, although with HCl there is a fall in immunity at 30' after fertilization.

3. The marked decrease in immunity "at cleavage" caused by the four agents employed seems to be explainable on the basis that all, in one way or another, *augment* beyond a certain point the increase in osmotic pressure normally necessary for cleavage. The results obtained seem therefore to be confirmatory of the position presented by the author elsewhere¹ that cleavage is due to the equalization (*Ausgleichung und Umformung*) of an uncompensated potential difference between osmotic pressure and surface tension, accompanied by electronic phenomena which cause constriction.

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